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Review

Fabrication of Polymersomes: A Macromolecular Architecture in Nanotherapeutics

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Abstract: In consideration of the issues of drug delivery systems, the artificial vesicle structures composed of block copolymers called polymersomes recently gained considerable attention. The possibility of tuning the mechanical parameter and increasing the scale-up production of polymersomes led to its wide application in healthcare. Bearing in mind the disease condition, the structure and properties of the polymersomes could be tuned to serve the purpose. Furthermore, specific ligands can be incorporated on the vesicular surface to induce smart polymersomes, thus improving targeted delivery. The synthesis method and surface functionalization are the two key aspects that determine the versatility of biological applications as they account for stability, specific targeting, degradability, biocompatibility, and bioavailability. A perfectly aligned polymer vesicle can mimic the cells/organelles and function by avoiding cytotoxicity. This supramolecular structure can carry and deliver payloads of a wide range, including drugs, proteins, and genes, contributing to the construction of next-generation therapeutics. These aspects promote the potential use of such components as a framework to approach damaged tissue while maintaining healthy environments during circulation. Herein, this article concentrates specifically on the drug delivery applications of polymersomes.

Keywords: polymersomes; nanoenabled drug delivery; targeted drug delivery; anticancer; chemotherapy

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1. Introduction

It is already evident that the delivery carriers are essential to transport the drug without disturbing its stability inside the body. This can be accomplished only when there is a complete understanding of the physiological conditions of disease as well as the features of such drug moieties. The pharmaceutical industry and research institutions spend enormous amounts of money and effort every year on improving therapeutic delivery systems. This involves the development of novel techniques and application of the newer formulation, such as nanostructures at the forefront of creating an efficient drug delivery system [1–3]. In recent decades, the field of drug delivery has seen a spectacular leap when nanomaterials have been introduced, which paved a new learning curve compared to bulk materials [4,5]. A nanomaterial has a larger surface area, greater potential, higher chemical reactivity, and conductivity than traditional material. It is possible to encapsulate different therapeutic agents or payloads in nanomaterials and to allow their content to be carried and released at the diseased site having specific physiological environments.

Cancer and bacterial infection pose great threats to the health and well-being of humans. A variety of mechanisms may lead to the ineffectiveness of antibiotics, including the production of enzymes that degrade antimicrobials, changes in target sites or membrane permeability, the transmission of biofilm, and enhanced active efflux mechanisms [6]. Similarly, the treatment relies on chemotherapy and tumor surgery in order to stop the spread of cancer and prevent it from recurrence, and as a result of the resistance to anticancer drugs, cancer chemotherapy faces a number of challenges. In the past few decades,

a variety of nanoplatforms including liposomes, hydrogels, niosomes, micelles, emulsions, polymersomes, nanospheres, and nanocomposites have been extensively investigated for targeted drug delivery [7–14]. Through endocytosis, these drug-loaded nano-platforms lead to increased drug accumulation in cancer cells by preventing efflux pumps or altered membrane permeabilities. The chemodynamic therapy, photodynamic therapy, and photothermal therapy are some alternative strategies for treating drug-resistant cancers too [15–18]. Auxiliary reagents, such as a photosensitizer, can sometimes be targeted to cancer tissues and damage the cells by producing ROS (Reactive oxygen species) or heat [19]. Moreover, these alternative techniques have also proven to be very effective in treating infections caused by bacteria. As a result, lipids and copolymers have the end-functional groups responsible for controlling the release of active substances, typically in response to physical and chemical stimuli to reduce potential damage caused to healthy cells around the diseased cell membranes [20].

Self-assembled amphiphilic block copolymers made of polymersome or polymeric vesicles are very promising nanostructures that appear to be highly stable, as biocompatible devices that have been extensively investigated for various biomedical applications including cancer theranostics and antibacterial therapy [21]. In general, polymersomes are composed of hollow membranes, similar to those found in cells or liposomes. The flexibility of polymeric segments, easy modification to attach specific functional groups, controllable particle size, hydrophilic cavities with high capacity, and multifunctional membrane structure make polymersomes ideal for drug delivery [22,23]. Since polymersomes are a membrane that is covered with abundant hydrophilic polymers, they are considered to be more stable than liposomes [24]. As an additional benefit of polymersomes compared to polymeric micelles, they are capable of loading hydrophilic drugs in the lumen at the same time as hydrophobic drugs on the membrane. Other than drug delivery and therapeutic potential, polymersomes also can act as biosensors [25]. Ghorbanizamani et al. were the first to report polymersomes based colorimetric assay for the detection of spike protein (Covid-19). The assay created using polymersomes, composed of poly-caprolactone copolymer and methoxy polyethylene glycol encapsulated with a dye called fuchsine, possessed high reproducibility and sensitivity, which is the major concern of detection techniques [26]. Meanwhile, this review summarizes the principles, synthetic routes, and recent developments of polymersomes for therapeutic applications.

2. Structure and Surface Modification of Polymersomes

The concept of polymeric vesicles was inspired by the phase characteristics of amphiphilic copolymers. There are numerous advanced features of polymersomes that make them the ideal alternative to liposomes, such as higher stability at biological pH, superior functionalization, tunable mechanical properties, the high choice for encapsulation of drugs, and attractive drug release profile, bioavailability, biodegradability, and cargo release through stimuli responsiveness. A polymersome is a hollow sphere with an aqueous core and a polymer shell made of diblock polymers (Figure 1) [27]. An AB type of diblock copolymer can be used to synthesize polymersomes where A is a hydrophobic type of diblock copolymers, and B is the hydrophilic type of diblock copolymers [28]. Additional to the AB types of polymersomes, it can also consist of triblock copolymers, where liner structures are formed from another hydrophobic block. It is possible to form polymersomes based on molecular weights (MWs) and chain length using amphiphilic block copolymers [29]. There is a wide range of controlled membrane thicknesses from 2 nm to 50 nm for polymersomes [30,31]. Compared to polymersomes with a thinner membrane thickness, a thicker membrane has demonstrated greater stability and rigidity, as well as greater efficacy in entrapping hydrophobic drugs. Reports produced by Zheng et al. include synthesis of polymersomes through RAFT polymerization (reversible addition fragmentation chain transfer) technique, crosslinking of pentaflurophenyl groups using diamine linkers, labeling fluorescein-5-isothiocyanate dye on the surface, characterizations, in vitro and in vivo analysis using glioblastoma cells and mice, respectively. The study

suggests that the prepared particle was stiff and accumulated well in brain tissues. It is also found that the modification in the crosslinker apparently reflected on the function and characteristics of the particle. Thus, this work intensified the importance of the mechanical properties of a particle in their biological applications [32]. In polymersomes, block copolymers self-assemble to form spheres, vesicles, cylinders, or elongated micelles based on the length distribution. Polymersomes are externally modified to increase their uptake by cell structures by forming selective interaction between the particle and cell membrane. Over the past several decades, it has been a challenging area to design biofunctional polymersomes that can be translated into clinical settings [33]. Nam et al. discovered that lipo-polymersomes (HLPs) could be made by mixing diblock copolymers such as poly(butadiene-*b*-ethylene oxide) with common natural lipids to form a compositional heterogeneous hybrid lipo-polymersome. Through thermally driven phase separation and cholesterol inclusion, lipid-rich domains are mixed and demixed from polymer-rich matrixes of hybrid vesicles. Cooling rate and lipid composition can control domain size and morphology. In addition to their biocompatible-measuring properties, composite membranes are also structurally stable and can be used for encapsulation-based technologies such as delivery and sensing applications, or the control of biochemical reactions [33].

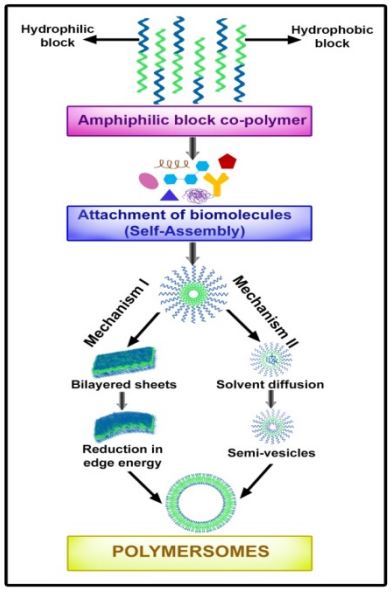


Figure 1. General process to obtain polymersomes.

It is necessary to take extra steps in order to form vesicles when polymersomes are conjugated with bulky ligands like antibodies for targeting. Purification, centrifugation, dialysis, and filtration are the most commonly used techniques, all of which have the potential to damage or alter vesicles in some way. Therefore, purifications and surface modifications are always challenging tasks for scientists. For the conjugation of polymersomes with antibodies and ligands, there are several different chemical reactions that have been widely used, such as click chemistry, biotin-streptavidin interactions, Michael addition, and EDC-assisted coupling [34]. The carboxylated polymersomes synthesis often generates intermediates with high hydrolysis affinity causing an issue. An additional amount of carbodiimide must be added to complete the reaction in order to stop the hydrolysis of o-acylisourea as an intermediate in carboxylated polymersomes synthesis [35]. When carbodiimide is present in excess, the colloidal stability of vesicles is altered. The problem can be overcome by using succinimide, which is more stable and has a lower hydrolysis affinity. To attach large biomolecules to polymersomes, amine groups can also be added to the polymersome surface. To accomplish this, bis-aryl-hydrazone bonds have been added to copolymers. As an additional approach to functionalize polymersomes, Michael addition reaction employed a thiol-maleimide coupling strategy. At near-biological pH, thiol-ether

bonds are formed due to the stability, greater efficiency, and selectivity of maleimide towards thiol. The synthesis route is one of the important aspects that affect the characteristic potential of a particle. Studies carried out by Travanut et al. studied the biocompatibility of the polymersomes synthesized through a versatile technique called as Passerini 3CR (3 component reaction). The results demonstrated that this technique could provide a facile synthesis of a particle with cytocompatibility and biodegradability, making them a potential drug carrier [36]. Similar work conducted by the same group intensified the importance of the synthesis route in the performance of a particle. The study included the fabrication of polymersomes using the same Passerini chemistry, encapsulating a doxorubicin drug to get an effective treatment against malignant breast cancer. The Passerini polymers synthesized via one-pot reaction featured greater therapeutic efficiency proving that Passerini chemistry allows synthesis for particles with better characteristics [37]. There is another method of modifying the surface of polymersomes by creating an interaction between a copper-catalyzed azide-alkyne cycloaddition and azide using the click chemistry approach. Among the most commonly used methods to target polymersomes is the ligation strategy that involves both biotin and streptavidin. Broz et al. designed polymersomes made up of triblock polymer functionalized with biotin to specifically target the A1 receptor of macrophages. The ligand biotin was functionalized onto the polymer using the coupling agent streptavidin. This particle served diagnostic and therapeutic purposes [38]. There are many advantages associated with this method, including its simplicity, the stability of the bonds, and the compatibility with antibodies and other targeting moieties that can be attached to the modified biotin-streptavidin or biotin-avidin copolymers.

3. Polymersomes as Drug Carrier

The loading of hydrophilic substrates into polymersomes can either be passive or active. A passive loading strategy involves adding the drug to both the aqueous phase and the inner water phase of the polymersomes [39]. This approach involves the diffusion of uncharged drug molecules across the outer structure to reach the core of vesicles. The low or high core pH accounts for the substrate to become charged. With this method, it is possible to achieve high encapsulation efficiency and stable retention of the drug molecules. In remote loading, polymersomes with thicker membranes may prove advantageous since they enable the substrate to diffuse rapidly [40]. Köthe et al. developed a novel method called DAC (Dual asymmetric centrifugation) to prepare polymersomes, where poly caprolactone and polyethylene glycol block copolymers were used as precursor molecules. The particle prepared showed high efficiency in drug loading of both hydrophobic and hydrophilic moieties. The method used no organic solvents and did not involve any post-processing steps, making it a facile process for the fabrication of polymersomes as the best suitable drug carrier [41]. Polymersomes can be encapsulated and decorated with targeting ligands by combining small and large molecules with a block copolymer. It may be possible to extend the release time of drugs when they are incorporated into vesicles by using biodegradable linkers that improve the retention of the drug. Walvekar et al. studied the potential role of polymersomes in the delivery of antibiotics against MRSA (Methicillin-resistant Staphylococcus aureus) infections. The particle was synthesized using a self-assembly technique, encapsulated with vancomycin drug, and conjugated with oleylamine- hyaluronic acid ligands. The in vitro study results indicated that this particle would act as a promising drug carrier for the treatment of bacterial infections [42]. Another study by Porges et al. also developed polymersomes for the treatment against antibacterial infections. Block copolymer-based polymersomes were fabricated and encapsulated with antibiotics for the treatment of Burkholderia infections. It was concluded that the developed polymersomes would serve as the best carrier for antibiotics [43]. A transmembrane channel protein can be incorporated into a polymersome membrane to increase its permeability for hydrophilic molecules and ions. It has been shown, for example, that in cascade reactions enzymes encapsulated in membranes containing channel proteins can avoid inhibiting factors and also maintain the ability to access the substrate. This has been demonstrated in the synthesis of

polymersomes with cytidine-monophosphate-N-acetylneuraminic acid and OmpF G119D (channel protein) to retain access to the substrate. Alibolandi et al. developed dextran based polymersomes for insulin delivery. The particle was prepared using the hydration method and encapsulated with insulin. The in vivo and in vitro study results demonstrated that the particle loaded with insulin significantly delivered the payload via an oral drug delivery system [44]. As part of more fundamental studies on membrane proteins, polymersome membranes had the ability to be tuned in their mechanical properties by adjusting the chemical composition, allowing for a better insertion of membrane proteins. The functionalization of transmembrane proteins is limited by the thickness of the membrane since most of the channel proteins are designed for only lipid membranes. PMOXA-*b*-PDMS-*b*-PMOXA model demonstrates that polymersome membrane conformations adapt to allow biopores smaller than hydrophobic membrane layers [45,46].

When polymersomes are made from long block copolymers, their membranes can have a thickness of up to 50 nanometers. Accordingly, compared to liposomes, polymersomes are theoretically capable of accommodating greater amounts of hydrophobic molecules [47]. Furthermore, thick membranes can lead to a slower release of hydrophobic substrates because the diffusional distances are much higher. When the vesicle is formed, the hydrophobic molecules or drugs occupy the organic part and incorporate it into the membrane. A 47% encapsulation efficiency was achieved by polymersomes made up of poly(trimethylene carbonate) and poly(L-glutamic acid) loaded with doxorubicin by nanoprecipitation at pH 10.5 [48]. A low diffusion distance may result in low drug retention when loading substrates. There is a risk of a high rate of drug release in polymersomes due to the narrow diffusion distance, despite their membrane thickness. A biodegradable polymer might further impair the release profile of the cargo in vivo, as a high polymer degradation rate could result in the destabilization of the membrane and an acceleration of the cargo release process [49]. Table 1 describes some more applications of polymersomes in drug delivery.

Table 1. Complies with recent advancements in polymersomes for drug delivery applications.

Scheme. No	Polymer Used	Target Moiety Or Payload	Method of Polymersomes Formation	Application	Ref.
1	Poly(lactic acid)-co-poly(ethylene glycol) (PLA-PEG)	Azobenzene	Solvent exchange method	Pancreatic adenocarcinoma	[50]
2	Amphiphilic triblock copolymer (PVBz-*b*-PEG-*b*-PVBz)	-	Solvent injection method	Intracellular drug release	[51]
3	Leukoadhesive Biocytin-terminated copolymer (PEO(1300)-*b*-PBD(2500)	Avidin, Sialyl Lewis X tetramer, anti-ICAM-1 antibody and fluorophores PZn_2	Self-assembling	Targeted drug delivery in inflammation	[52]
4	PEG-*b*-PCL	Hypericin, peptides, and ceftriaxone	Dual asymmetric centrifugation	Biomedical applications	[41]
5	Poly(ethylene oxide)-*b*-poly(acrylic acid)-*b*-poly(*N*-isopropylacrylamide)	FITC-dextran	Self-assembled	Smart carriers for pDNA, siRNA, peptides, and proteins	[53]
6	Poly (*o*-nitrobenzyl acrylate) and Poly (*N*,*N*′-dimethylacrylamide)	Hydrophilic and hydrophobic drugs	Self-assembled	Photoresponsive drug carrier	[54]
7	Polymer of Didodecyl substituted *o*-nitrobenzyl	Folic acid and doxorubicin	Self-assembled	Targeted photoresponsive drug delivery	[55]
8	*N*-(2-hydroxyproyl) methacrylamide (HPMA)-analog	Fluorescent dye and a hydrophilic moiety	Solvent switching method	Hydrophilic cargo like siRNA	[56]
9	Triphenylphosphonium cation conjugated PLA-PEG	Doxorubicin	Self-assembled	Anticancer drug delivery to mitochondria	[57]
10	Pluronic/Poly(lactic acid)	Biotinylated Transferrin, folic acid and paclitaxel	Self-aggregation	Dual targeting drug delivery	[58]
11	Pluronic F127-PLA/D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS)	Folate conjugated and paclitaxel loaded	Self-aggregation followed by dialysis	Oral anticancer delivery	[59]

4. Polymersome-Based Targeted Anticancer Drug Delivery

It has been over a decade since multiple chemotherapeutics were developed for the treatment of cancer, but in spite of this there are still many challenges in the field of chemotherapeutics like less selectivity, physiological barriers, half-life, and poor bioavail-

ability [60]. Additionally, due to the damage to the liver and kidney caused by cancer therapeutics, chemotherapy may even be discontinued in the course of treatment. Moreover, resistance to anticancer drugs is a major concern because it occurs due to abnormal expression of drug-efflux transporters, which prevents the intracellular transport of cancer therapeutics. This is because drug carriers can improve the bioavailability of anticancer drugs, enhance permeability and retention (EPR) effect, reduce side effects, and provide efficient delivery of chemotherapeutics bypassing the biological barriers [61]. Since polymersome-based drug delivery systems are characterized by a stable structure like a cell with a membrane and lumen that are size-controlled, they have been investigated as smart cancer therapeutics nanocarrier systems [62]. It is possible for polymersomes to encapsulate both hydrophobic and hydrophilic anticancer agents in their lumens or membranes. Furthermore, the polymers can be functionalized with ligands complementary to receptors on the target site as well as with ligands that are sensitive to environmental responsiveness, so that they can deliver drugs precisely and release them in response to stimuli [63]. Polymersomes offer an excellent opportunity to target postsurgical premetastatic niches and microresiduals, resolving the challenges of poor therapeutic delivery [64]. Several studies have demonstrated the synergistic antitumor effect of combination chemotherapy and immunotherapy for cancer. After proper combination with chemotherapy, immunotherapy can significantly improve response rates and efficacy. A study by Japir et al. suggests that tumor-dilatable polymersome nanofactories that are capable of long-term intratumoral retention may represent a promising strategy to enhance enzyme prodrug chemo-immunotherapy using polymersomes [65].

As a result of targeted delivery systems, the target markers in cancer cells that are overexpressed or unique are targeted [66]. A number of cancer targets have been identified, and a number of targeting ligands have been employed in the development of polymersome-based targeted delivery systems, including folate, antibodies, etc. [67]. For example, Polymersomes containing folate are able to target cancer cells by means of endocytosis through the folate receptor. In order to survive a complicated physiological environment and provide efficient targeting capability, these targeted ligands have been conjugated primarily with polymersomes by covalent bonding [68,69]. The folate molecule contains two carboxylic acid groups, which greatly influence polymersome reactivity. In cancer, antibodies may bind specifically to antigens that are over-expressed or those that are cancer-specific. A number of studies had been carried out in the early stages to achieve targeted therapy by conjugating antibodies onto anticancer drugs. Lee et al. conducted in vitro study of anticancer drug delivery to a specific site of breast cancer cells by conjugation of antibodies. Diblock co-polymeric substances containing polyethylene glycol and poly (lactic co-glycolic acid) were synthesized using the solvent evaporation technique. During this fabrication, indocyanine green and doxorubicin were encapsulated in the polymer molecules. Since breast cancer cells overexpress HER2 gene, a complementary binding agent-anti-HER2 antibody was conjugated through the carbodiimide crosslinking method. The experimental results showed that the cell death rate was much higher in the polymersomes encapsulated with the dual drug compared with a single drug dosage. Hence this study indicates that drugs encapsulated in polymeric structure readily reduced the chemotoxicity and increased the therapeutic effect [70]. Clinical trials or even marketing approvals have already begun for several antibody-drug conjugates for cancer treatment. However, there is a low ratio of drug to antibody in the antibody-drug conjugate, which means excessive antibodies are consumed when preparing antibody-drug conjugate, resulting in reduced drug-to-antibody ratios. Alternative options include conjugating antibodies to polymersomes encapsulated with drugs [71,72]. The development of polymersome-based targeted delivery systems has currently been accomplished using several types of antibodies, including anti-EGFR antibodies (cetuximab, trastuzumab), anti-CD44 antibodies, anti-PSCA antibodies, and anti-EpCAM antibodies, among others. The monoclonal antibody AM6 was used to target breast cancer cells. Khanna et al. recently reported that the nanocarrier prepared using poly (lactic co-glycolic acid) encapsulated with paclitaxel

drug showed better targeting to breast cancer cells due to the surface functionalization of AM6 antibody. The antibody was conjugated using the reaction chemistry between thiol and maleimide. The results obtained from in vitro and in vivo determine that the antibody-conjugated particle actively targeted perlecan in cancer cells and significantly helped to facilitate the drug release [73].

In cancer cells, short peptides of specific sequences bind to abnormally overexpressed proteins (integrin or transferrin) membrane and enhance their ability to penetrate the cells' membranes. These peptides were found to have significant abilities to target cancer cells and could be conjugated with polymersomes to provide their ability to target. WREAAYQRFL (tumor-homing peptide) was used by Oz et al. to design an integrin-targeted polymersome [74]. This study showed that the peptide was capable of recognizing and binding to $\alpha v\beta 3$ integrin, which is pivotal in tumor growth, spread, and metastasis. Breast cancer cells typically overexpress this integrin. Thiol-ene click chemistry was used to chemically link the targeting peptide to a diblock copolymer. The peptide-conjugated polymers were then assembled to form polymersomes to encapsulate the anticancer drug doxorubicin during the self-assembly process. The use of tetraiodothyroacetic acid, triphenylphosphonium cation, and biotin has also been considered for targeted delivery solutions based on polymersomes [75]. By amination between the amino group and p-anisic acid on polymer chains, anisamide can be incorporated into polymersomes for its affinity to overexpressed sigma receptors in the tumor region. It is also important to note that these polymersome-based targeted delivery systems have the potential to target not only the overexpressed receptors found at tumor sites but also some tissue constituents, like the divalent calcium ions found in hydroxyapatite that accounts for bones formation, which can serve as potential targeting element. Since it was found that folate receptors are abundant in the gastrointestinal tract, oral delivery of anticancer agents is now the best choice of treatment. Hence, Pan et al. worked on the fabrication of polymersome-based carriers suitable for oral drug delivery systems. The polymersomes were prepared with polylactic acid and pluronic F127 and decorated with folic acid ligands. The particle was loaded with paclitaxel and was added to the particle to improve the absorption of the drug Vitamin E TPGS. The in vitro and in vivo results proved that the particle exhibits higher cellular toxicity compared with free drugs. The bioavailability was readily increased due to the specific interaction between the folate receptor and folic acid ligand. Consequently, this particle can be effective in the case of oral delivery [59].

The physiological barriers such as the blood–brain barrier (BBB) and blood–tumor barrier (BTB) remain as major obstacles to the treatment of brain tumors, including gliomas. In order to overcome both the BBB and the BTB, a dual-targeted or multitargeted polymersome delivery system is highly regarded. Research conducted by Figueiredo et al. concluded that overexpression of protein-1 related to low-density lipoprotein in BBB could serve as a target for efficient delivery. The polymersomes were developed with diblock copolymers encapsulated with doxorubicin and conjugated with angiopep-2. The angiopep-2 can actively target the over-expressed protein in BBB. The in vitro study results carried out on glioblastoma cells showed higher cytotoxic effects compared to free drugs. Thus, polymersomes conjugated with angiopep-2 can provide sustained release of the drug at its targeted site [76]. It was discovered by Chen et al. that an efficient polymersome delivery system attaching des-octanoyl ghrelin and folic acid effectively delivers the anticancer drug to brain tumors through dual-targeted polymersomes. The protein des-octanoyl ghrelin travels in the direction of blood to the brain, which may enhance drug-encapsulated polymersome transport across the BBB [77]. Folic acid could directly bind to folate receptors on cancer tissues after traversing the BBB to facilitate receptor-mediated endocytosis for drug concentration enhancement in the cancer cells. Polymersome-based delivery may improve delivery efficacy over conventional delivery systems, but still, there is a requirement to promote rapid drug release within tumor sites to improve therapeutic efficacy. In this regard, stimuli-responsive smart polymersomes can be more effectively used as a means of drug delivery system with controlled release profiles in response to stimulation [78].

5. Smart Polymersomes-Based Delivery Systems

There are many advantages to the intelligent delivery system, such as the delivery of the drugs effectively with rapid release at the tumor site reducing the probability of drug release in the bloodstream or surrounding healthy tissues [17,79]. The microenvironment of tumor tissues differs quite a bit from that of normal tissues, including pH, hypoxia, and overexpression of enzymes (Figure 2) [80–82]. To construct polymersome-based smart delivery systems, it is necessary to take advantage of the specificity of the microenvironment in tumor tissues to design and build environment-responsive polymers (Table 2). The response to external stimuli, such as ultrasound, light, or magnetic fields, can also be used. The smart polymersome-based delivery is classified into three categories depending on their source of stimulation and function. The chemical stimuli include the pH of the microenvironment, redox, reactive oxygen species, and ions, whereas the physical stimuli include temperature, heat, light, magnetic field, and ultrasound. Ultrasound waves will help to improve the release of the drug from the carrier into the target cell efficiently. Zhong et al. fabricated nanobubble carrier system using poly (lactic co-glycolic acid). The carrier was loaded with paclitaxel drug via double emulsion and conjugated with herceptin antibody via carbodiimide coupling reaction. The in vitro and in vivo study experiments showed that the herceptin decorated nanobubble reacted well to the ultrasound and promoted the targeting and release. This study concluded that this particle, when combined with ultrasound, could function as theranostic agent in image-guided therapy [83]. Other than these two, there are some biological stimuli like proteins, enzymes, and bioactive molecules like ATP. It has been reported that polymersomes that are pH- and redox-sensitive are most commonly found.

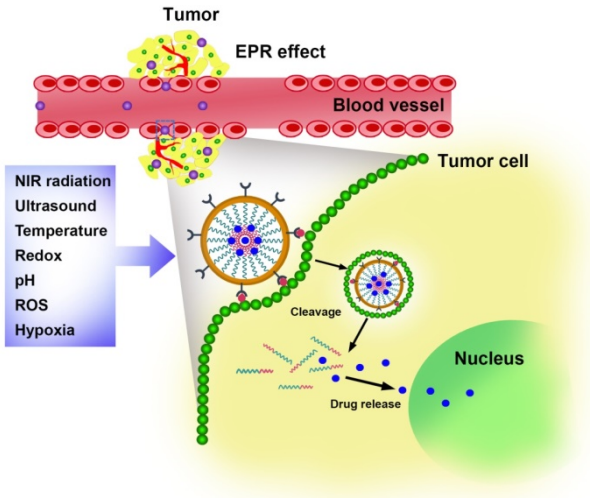


Figure 2. Stimuli responsive characteristics of polymersomes.

Changes in pH can affect the structure or properties of pH reactive polymersomes. The introduction of pH-responsive groups is one way to achieve pH-responsive ionizability. Under alternant change in pH, the polymers can reversibly modify their pH-responsive properties, which can influence the accelerated release of the drug encapsulated within [84]. In addition to this approach, it is also possible to attach pH-sensitive bonds into polymer, after which the release of drugs in polymer chains can be accomplished by cleavage in chemical bonds or by pH-induced degradation of main chains and side chains. Therefore, polymersomes are stable at physiological pH; hence the structure or properties of polymersome can be altered to trigger the release of drugs in the pH of the microenvironment, which is lowered (acidic conditions). Albuquerque et al. developed block copolymer encapsulated with doxorubicin via microfluidic technology. This method produced monodispersed polymer and was further characterized using hi-end photophysical tools. The in vitro results

showed that doxorubicin-encapsulated polymer possessed pH responsiveness towards tumor microenvironment and delivered the drug precisely [85].

For cells to proliferate and function properly, glutathione plays a critical role, and due to the rapid proliferation of cancer cells glutathione concentrations are at least four times higher in cancer cells than in those of healthy tissues [86]. During exposure to a reductive condition like a high glutathione environment, the disulfide bond cleaves to produce free thiol. In order to trigger glutathione-induced drug release, redox-responsive polymersomes were developed [87]. Therefore, through disulfide bonds, anticancer drugs can be covalently conjugated to amphiphilic polymers. Upon delivery to cancer cells, glutathione cleaves the disulfide bonds to release the anticancer drugs to the cancer cells. As a result of this method, we are able to produce polymersomes containing anticancer drugs that have a high drug loading capacity to avoid the release of drugs in circulation. In addition, another approach is to design amphiphilic polymers that are composed of disulfide bonds along their main chains or on their side chains [88]. The cleavage of disulfide bonds in an environment with high glutathione concentrations produces alterations in hydrophobicity or dismantling of polymer structures, which results in the rapid release of payload as a result of the cleavage of disulfide bonds. Additionally, external physical stimuli can be precisely applied to specific tumor sites to minimize unnecessary side effects [89]. As a result of temperature-sensitive polymersomes, the chemical properties and morphology respond to it and release the drug rapidly. Polymersomes that respond to temperature have been developed using a variety of temperature-sensitive polymers [90]. As a noninvasive stimulus, ultrasound and magnetic fields have also been considered as promising possibilities.

Polymersomes that respond to biological stimuli have also been developed using enzymes and ATP. Taking advantage of the microenvironment, the particle can be designed in a way to respond to the condition. The hypoxic surrounding is one of the common characteristics possessed by tumor cells that will contribute to the growth and relapse of tumors. This condition also limits the entry of drug carriers into solid tumor tissues. Designing a carrier, which could respond to hypoxia, will facilitate the entry and delivery. According to this hypothesis, Kulkarni et al. designed an echogenic particle that can respond to hypoxic stimuli. Polymersomes were fabricated with polyethylene glycol and poly lactic acid. The particle was then functionalized with azobenzene and peptide- iRGD for hypoxia responsiveness and tissue penetration, respectively, also encapsulating gemcitabine for treating pancreatic adenocarcinoma. The in vitro and in vivo results demonstrated that the polymersomes penetrated well into the solid tumor and delivered the drug efficiently. The echogenicity of the particle helped in imaging the study model under the ultrasound technique. Thus, it is concluded that the polymersomes designed above served as drug carriers and monitored the delivery simultaneously [50].

Table 2. Recent research evidence based on the development of stimuli-responsive polymersomes.

Scheme No.	Polymer	Payload	Stimuli Response	Application	Ref.
1	PEO-*b*-PCSSMA copolymer	Doxorubicin	Dual responsive (Light and reduction)	Synergistic loading and programmed release of therapeutics	[91]
2	Poly (ethylene glycol) monomethyl ether-*b*-methyl methacrylate-ran-2-(dimethyl amino) ethyl methacrylate mPEG-*b*-(PMMA-ran-PDMAEMA)	Curcumin, 2-naphthole, paclitaxel, and ampicillin sodium salt	pH-responsive	Intermolecular drug release interactions	[92]
3	Poly(ethylene oxide)-*b*-poly(2-((((5-methyl-2-(2,4,6-trimethoxyphenyl)-1,3-dioxan-5-yl)methoxy)carbonyl)amino)ethyl methacrylate) (PEO-*b*-PTTAMA)	Nile red Doxorubicin	pH-responsive	Targeted combinational therapeutic drugs	[93]
4	Poly(2-(methacryloyloxy)ethyl choline phosphate)-*b*-poly(2-(diisopropylamino)ethyl methacrylate) (PMCP-*b*-PDPA)	Doxorubicin	pH-responsive	Targeted intracellular delivery system	[94]

Table 2. *Cont.*

Scheme No.	Polymer	Payload	Stimuli Response	Application	Ref.
5	Methoxyl poly(ethylene glycol)-*b*-poly(*N*-isopropylacrylamide)-*b*-poly[2-(diethylamino)ethyl methacrylate-co-2-hydroxy-4-(methacryloyloxy)benzophenone]	Doxorubicin, paclitaxel	pH-responsive Temperature	Promising drug carriers for tumor combination chemotherapy	[95]
6	Poly(3-methyl-*N*-vinylcaprolactam)-block-poly(*N*-vinylpyrrolidone) (PMVC-PVPON)	Doxorubicin	Temperature	Tumor targeting and next-generation drug carriers	[96]
7	Poly(ethylene glycol)-*b*-poly(*N*,*N*-diethylaminoethyl methacrylate) (PEG-*b*-PDEAEM)	Gold nanorods, doxorubicin	pH-responsive, NIR-irradiation	Photothermal therapy and targeted drug delivery vehicles	[97]
8	Poly(ethylene oxide)-block-poly(2-(diethylamino)ethyl methacrylate)-stat-poly(methoxyethyl methacrylate) [PEO-*b*-P(DEA-stat-MEMA)]	Doxorubicin	Ultrasound	Chemotherapeutic efficiency	[98]
9	Poly(*N*-isopropylacrylamide)-dox-polyethylene glycol)-2,4,6-trimethoxy benzylidene pentaerythritol carbonate (PNIPAM-DOX-PEG-PTMBPEC)	Doxorubicin	pH-responsive, Temperature	Development of Cancer Therapy	[99]
10	Poly(*N*-vinylcaprolactam)$_{10}$-*b*-poly(dimethylsiloxane)$_{65}$-*b*-poly(*N*-vinylcaprolactam)$_{10}$	Doxorubicin	pH-responsive, temperature	Delivery of hydrophobic and hydrophilic anticancer therapeutics and controlled drug delivery system	[100]
11	Poly[2-(dimethylamino) ethyl methacrylate]-block-polystyrene (PDMAEMA-*b*-PS)	Ascorbic acid, cationic EPR probe CAT1	Ph-responsive, Temperature	Gene delivery and Nanoreactors	[101]
12	Amphiphlic poly(ethylene glycol)-block-poly(β-aminoacrylate)-block-poly(ethylene glycol)	Doxorubicin and photosensitizer IR-780	NIR responsive	Chemotherapy, Photothermal, and photodynamic therapy	[102]
13	poly(ethylene glycol)$_{113}$-b-P-(CPT methacrylate monomer$_{0.48}$-co-2-(pentamethyleneimino) ethyl methacrylate$_{0.52}$)$_{92}$ PEG$_{113}$-*b*-P(CPTKMA$_{0.48}$-co-PEMA$_{0.52}$)$_{92}$	Camptothecin	ROS-responsive pH-responsive	Starving therapy, chemodynamic therapy, and chemotherapy	[103]
14	Poly(propylene sulfide)$_{20}$-bl-poly(ethylene glycol)$_{12}$ (PPS$_{20}$-*b*-PEG$_{12}$)	Zinc phthalocyanine (ZnPc), Doxorubicin	ROS NIR irradiation	Chemo-photodynamic therapy	[104]

It is possible for polymersomes with dual responses to more complex intracellular environments. To demonstrate the concept of dual-responsive polymersomes, a series of polymersomes with dual-triggered release capability has been developed. Through reversible addition–fragmentation chain transfer polymerization (RAFT), Zhou et al. have constructed dual, thermo-, and pH-responsive polymersomes containing a self-assembled triblock polymer. A layered membrane was present in the dual-responsive polymersomes, allowing the permeability of the membrane to be adjusted. Additionally, it was demonstrated that the polymersomes responded to temperature changes based on pH. In the fabricated polymersomes, doxorubicin hydrochloride (DOX) and paclitaxel (PTX) had the potential to be encapsulated in a hydrophilic-hydrophobic pair. In order to independently control the release of DOX and PTX, the membrane permeability was triggered by temperature and pH changes. The polymersomes encapsulated with DOX and PTX could work synergistically in simulated tumor microenvironments [95]. With the same RAFT technique, Rodriguez et al. developed polymersomes using amphiphilic block copolymers. The particle was encapsulated with an anticancer drug, doxorubicin, and gold nanorods. The particle possessed dual stimuli responsiveness of pH and NIR irradiation. This resulted in higher cytotoxicity due to an increased release rate at the site [97]. Khan and the others synthesized three types of polymers that were both reductive and acidic using poly(mPEG-SS amino) (*N*,*N*-diisopropylethylenediamino)phosphazenes] (PPDPs). Thereafter, the PPDPs were allowed to self-assemble into polymersomes loaded with hydrophilic or hydrophobic anticancer drugs in high loading content with higher encapsulation efficiency. As a result of the polymersomes made of PPDPs, an anticancer drug was released in response to reductive/acidic stimuli [105].

6. Engineering of Large-Scale Production of Polymersomes

In medical and biotechnological applications, polymersomes are expected to be acceptable carriers and universal reaction compartments due to their increased stability. In order to put polymersome technology into practice at the industrial level, it is imperative to develop controlled and cost-effective methods to produce large quantities of vesicles [106]. There are a couple of basic requirements that must be met as part of a scale-up strategy, among them the use of the same material for both the model scale and the larger scale processes. Therefore, it is critical to maintain physicochemical parameters influencing crucial material characteristics at both scales. Additionally, the process equipment needs to be linearly scaled up to maintain geometrical similarity. Finally, scale transfer criteria specific to a given process need to be identified and maintained. Poschenrieder et al. have reported the production of polymersomes using an amphiphilic triblock copolymer from 12 mL to 1.5 L controlling the process engineering parameters. The produced polymeric vesicles showed monodispersity with a hydrodynamic diameter of 180 nm, and the polydispersity index was maintained at <0.2 [106]. Rameez et al. have reported on the low-pressure extrusion procedure for the synthesis of polymersomes on a large scale. By continuously extruding hollow fiber membranes with pore diameters of 200 nm, vesicles were produced. A monodisperse empty polymersome with a particle diameter of less than 200 nm was formed [107]. It can be stated that polymersomes offer an additional advantage over liposomes due to their large-scale production.

7. Conclusions and Future Perspective

The progress in the development of polymersomes as nanotherapeutic platforms implies their importance in future pharmaceuticals. The attractive characteristics of polymersomes enabled the researchers to work with them in all possible ways, including drug delivery and imaging. This review, with special attention, described the potential application of polymersomes in the delivery of chemotherapeutics. The microenvironmental condition can be taken advantage of by fabricating particles that could specifically target the tumor tissues and release the drug in a slow and sustained manner. Ligands complementary to the receptors in the cancer cells are designed and decorated on the polymersome to enable site-specific drug delivery. In-depth knowledge on the biological interactions between the particle and cellular targets needs to be gained to advance in medical applications. However, recent reports suggest that they possess minimal toxicity as they enter the distribution step. A comprehensive and systematic study is strongly recommended to further minimize the adverse effects and thereby broaden the application of this technique by comparing it against liposomal or other stable vesicle models.

Undeniably, the design and development of new copolymers will show the way for the fabrication or engineering of polymersomes with the desired properties for integrating a variety of payloads into them. Clinical trials have actually been conducted with very few polymersome-based systems so far. Upscaling most polymersome preparation techniques is difficult and reproducible, which limits their application in clinical practice. For the evaluation of the biosafety of nanocarriers, it is essential to understand the pharmacokinetic and biodistribution parameters in vivo. Until now, little research has been done on the pharmacokinetics of polymersomes, as well as on their biodistribution. A primary consideration for practical applications of polymersomes should be their safety, and it must be evaluated whether polymersomes are safe on a long-term basis and if they are immunogenic in order to enhance this strategy further. There is a need for more research comparing polymersomes with therapeutic liposome controls, whose clinical safety profile is well established. This would help determine how effective polymersomes are in loading drugs, therapeutic efficacy, and pharmacokinetics, as well as biodistribution of drugs. Novel strategies for delivering therapeutic loads into tumor cells could be delivered by developing highly stable polymersomes with efficacies.

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